Plate having at least one well for holding chemical and/or biochemical and/or microbiological substances and method of producing the plate

The invention relates to a plate having at least one well for holding chemical and/or biochemical and/or microbiological substances, the well having an interior chamber, which is defined by its inner surface, and a wall, the outer side of which, remote from the inner surface, comprises a heat-exchange surface which can be brought at least in part into thermal contact with a temperature-control substance.

The invention relates also to a method of producing a plate having at least one well for holding chemical and/or biochemical and/or microbiological substances.

Such plates are obtainable from a number of suppliers and are known as microtest plates or microtitre plates.

The known plates have a rigid base which is surrounded on all sides by an upright rim. In the base, which is more than one millimetre thick, there are made, from above, wells which are arranged in rows and columns. On its underside remote from the wells, the base of the known plate is flat. The known plates are available with different well volumes, which are generally between a few hundred microlitres and a few millilitres.

It is known to cover the rim of the plate with a lid or with an adhesive film in order to protect the wells and the interior chamber defined by the rim from any kind of contamination.

The plates, which consist of polystyrene or polyvinyl chloride, are used for keeping solutions at a constant temperature. This is carried out either for storage purposes or to perform a reaction at a certain temperature. For that purpose, the plates - after the solutions have been introduced into the wells – are covered with the lid and either placed in a refrigerator for storage or in an incubator which is generally set to 37°C.

For solutions the reactions of which have to be controlled by frequent and rapid temperature changes, the known plates are not suitable because the cooling in the refrigerator or the heating in an incubator takes much too long.

Although it is known to control the temperature of the plates by means of their flat underside, in that case too changing the temperature of the samples takes up to several minutes.

Also known are plastics reaction vessels that taper conically to a point and have a snap-on lid or a screw-on lid. The known plastics reaction vessels are generally several centimetres tall and have an external diameter in the region of from 12 to 18 mm. When a large number of samples or solutions is to be processed, the amount of space and time required for handling the large number of reaction vessels is correspondingly great.

The temperature of the substances contained in the known reaction vessels is changed by controlling the temperature of the reaction vessels by means of their outer side. This is effected, for example, by immersing the reaction vessels in water baths of different temperatures. It is also known, however, to provide temperature-controlled metal blocks with bores for the reaction vessels, the temperature of which is then controlled by the contact with the walls of the bore. The heat transfer can be improved by filling the bores with water or oil.

The temperature of the samples can be changed by placing the reaction vessels into other water baths or metal blocks which are set to the new desired temperature.

The substances assume the new temperature only slowly, because the plastics reaction vessels have a thick wall through which only poor transport of heat is possible. The thick wall is necessary for production-related reasons and on account of the necessary mechanical strength of the reactions vessels which are handled individually. For example, the reaction vessels are inserted into centri-

fuges which rotate at high speed. The mechanical stresses that occur during the rotation are a reason for the stable thick wall of the plastics reaction vessels.

Many of the new chemical, biochemical or microbiological methods, however, require the reaction solutions to be brought to different temperatures as quickly as possible in the course of an experiment. Such reactions often have to pass cyclically through a specific temperature profile which may consist of a plurality of heating and/or cooling steps. The yield and efficiency of the reactions depends on the rate of temperature change in the solutions used. Especially in the case of enzymatic processes using nucleic acids, a rapid change between high temperatures for denaturing the nucleic acids and low temperatures for starting the reaction is required.

The invention is therefore based on the problem of developing a plate of the kind mentioned at the beginning to the effect that the above-mentioned disadvantages are avoided. In particular, it should be possible to change the temperature of a number of samples rapidly with simple handling.

That problem is solved according to the invention by the at least one well having a heat transmission value that is greater than 5×10^{-4} W/(K mm^3) and to which the following relationship applies:

$$\frac{A \cdot \lambda}{V \cdot x} = W$$

in which A is the size of the heat exchange surface, λ is the thermal conductivity of the material forming the wall, V is the volume of the interior space of the well, x is the wall thickness of the wall measured between the heat-exchange surface and the inner surface, and W is the heat transmission value.

The problem underlying the invention is in this way completely solved. Because, in the novel plate, the heat transmission value is greater than 5×10^{-4} W/(K mm^3), faster heat transport into and out of the well through the wall is possible. Depending upon the material chosen for the plate and the required volume of the

well, the person skilled in the art can now choose the size of the heat-exchange surface and the wall thickness of the well while observing the above-mentioned relationship. A large number of wells per plate can be provided, so that simply handling of, for example, reaction solutions that are to be processed simultaneously is possible.

On the other hand, for plates having the same heat exchange surface area and the same well volume, this means, for example, that a plate made of a material having 10 times better thermal conductivity but a 10 times thicker wall thickness has the same heat transmission value as a plate having poorer thermal conductivity and a thinner wall.

In a preferred configuration of the plate according to the invention, the wall thickness is approximately equal at least over the entire heat-exchange surface.

That measure has the advantage that the substances contained in the well are heated or cooled uniformly from all sides, which prevents the formation of troublesome temperature gradients in the substances.

In that exemplary embodiment, it is especially preferable for the heat transmission value to be greater than $1x10^{-3}$ W/(K mm^3).

That measure makes it possible for heat to be transported even more quickly through the wall of the well, which results in significantly better reaction results for the substances contained in the wells.

In that exemplary embodiment, a further advantage is obtained if the heat transmission value is greater than $3x10^{-3}$ W/(K mm^3).

By virtue of that measure, which provides even greater acceleration of the heat exchange, it is possible perform in the wells reactions that have to be controlled by temperature changes in the range of seconds. For example, it is possible to

heat or to cool an aqueous solution of 50 microlitres volume by about 60°C in less than 20 seconds.

Furthermore, it is advantageous for the plate to be made integrally with the well.

That measure has the advantage that the novel plate is simple to produce, because the wells do not have to be attached to the plate.

In that exemplary embodiment it is especially preferable for the plate to be made of plastics.

As a result of that measure, the plate has only a very low weight and is economical to produce, so that it can be conceived as a disposable or single-use article.

A further advantage is achieved in that exemplary embodiment if the plastics is thermally deformable.

As a result of that measure, it is possible to produce the plate in a quick and cost-effective manufacturing process, for example in a deep-drawing process.

In that exemplary embodiment, it is also preferable for the heat-exchange surface to be arranged on the plate below its underside.

That measure has the advantage that the heat-exchange surface for the temperature-control substance in question is readily accessible from below the plate. For example, the plate can thus be inserted from above into a water bath or into a metal block having a counter-surface for the heat-exchange surface.

In that exemplary embodiment it is also preferable for the well to be in the form of a beaker-like protuberance, the outer side of which is, at least in parts, the heat-exchange surface.

That measure makes it possible to accommodate the protuberances, for example, in corresponding recesses of a metal block thermostat, so that on the one hand the novel plate is held mechanically and on the other hand the heat-exchange surface extends over the entire extent of the outer side of the well, which results in a large and readily accessible heat-exchange surface.

In that exemplary embodiment it is, furthermore, preferable for the beaker-like protuberance to have a hollow-cylindrical upper portion joined to the underside of the plate and, formed integrally therewith, a hemispherical hollow lower portion.

The advantage of those measures is that the counter-surface to the heat-exchange surface can be produced in a metal block thermostat in a simple manner by means of a cylindrical blind bore having a rounded base. The geometrically simple shape of the outer side of the well provides a reproducible good heat transfer between the counter-surface in the metal block thermostat and the heat-exchange surface.

It is also preferable in that exemplary embodiment for the volume of the interior space to be less than 200 mm^3 and preferably between 10 and 100 mm^3.

Because most chemical and/or biochemical and/or microbiological reactions take place in that volume range, the plate according to the invention is especially well suited to such experiments as a result of that measure. In those experiments, the wells of the novel plate are at least filled to the extent that the solutions they hold are able to regulate the moisture content of the air volume above the surface of the liquid without any appreciable changes in their own volume.

In a development of that exemplary embodiment, in the novel plate the lower portion has a radius of between 2 and 6 mm and the wall thickness of the well is less than 0.2 mm.

As a result of that measure, which is very simple to achieve from a production engineering standpoint, the heat transmission value according to the invention,

which is greater than $5x10^{-4}$ W/(K mm^3), is achieved even in the case of plates made of plastics films, which tend to have poor thermal conductivity.

In that exemplary embodiment, it is also preferred for the wall thickness to be less than 0.08 mm.

As a result of that simple reduction in the wall thickness, the heat transmission value is advantageously significantly increased, which results in better heat exchange and accordingly in a more rapid change of temperature of the substances present in the well.

A further advantage is achieved in this exemplary embodiment when the plate is made of polycarbonate.

As a result of that measure, the plate is on the one hand provided with sufficient mechanical strength and on the other hand the wells can be formed, for example, by means of a deep-drawing process which is advantageous from the production engineering standpoint. Furthermore, the wells, which are produced from polycarbonate, are advantageous reaction vessels for most chemical and/or biochemical and/or microbiological reactions, because polycarbonate is inert in that respect.

That embodiment is advantageously developed by producing the plate from a polycarbonate film, the thickness of which is less than 0.5 mm.

That measure offers advantages from the production engineering standpoint in the respect that such polycarbonate films can be obtained ready-made. Forming the wells in such polycarbonate films also results in an advantageously small wall thickness, which in turn leads to a high heat transmission value.

It is also preferable in that exemplary embodiment for the plate to have a number of wells that are identical to one another.

That measure, known *per se*, offers the advantage that a large number of reactions can be carried out in parallel in the same plate, provided that all samples have to be subjected to the same temperature profile.

In that exemplary embodiment it is preferable for the wells to be arranged in rows and columns that are spaced equal distances of about 10 mm apart from one another.

That measure has the advantage, likewise known *per se*, that the wells can be filled and emptied using multiple pipettes, which makes the work involved in experiment preparation considerably easier.

A further advantage is achieved in that exemplary embodiment by providing the wells with a lid for closing its openings.

As a result of that measure, the wells can be protected from dirt and dust in a manner known *per se* after the substances have been introduced.

A further advantage is obtained in that exemplary embodiment when each region of the lid covering the opening of a well can be joined to the plate by means of a seam extending around the opening in question.

As a result of that measure, each well is subsequently provided as it were with its own lid by means of which the well is gas-tightly closed. Particularly in the case of small volumes of the interior spaces of the wells, the problem of evaporation occurring at high temperatures as well as the problem of condensation occurring at lower temperatures is thereby completely eliminated. The volume of liquid enclosed in the wells also does not change in the event of extreme and frequent temperature changes between high and low values.

That exemplary embodiment is developed especially advantageously by likewise producing the lid from a plastics film, especially from the same material as the plate.

As a result of that measure, it is especially simple to apply the seams between the lid and the plate. For example, the lid film can be bonded to the plate in the region of the seam under pressure and/or heat.

In an advantageous development of that embodiment, the seam in question gas-tightly seals the respective well.

As a result of that measure, even when small amounts of fluid are accommodated in the wells, no evaporation and/or condensation problems arise, even when the temperature of the wells is frequently changed between high and low temperatures.

An especially advantageous method of producing the plate from plastics is characterised in that a thermally deformable plastics film having a thickness of less than 0.5 mm and a thermal conductivity greater than 0.1 W/(K m) is laid, underside down, on top of a shaping block which comprises beaker-like depressions which are open at the top and the interior spaces of which have a volume of less than 200 mm^3; a stream of hot gas is directed from above onto the upper side of the applied film in succession in the region of each beaker-like depression for a certain time period; and the stream of hot gas has a fixed temperature in the vicinity of the melting temperature of the film.

In that method, the simple combination of the thermal conductivity of the film, the thickness of the film and the volume of the beaker-like depressions, has the effect that, as a result of the wall thickness of the wells produced during deep-drawing, the above-mentioned relationship for the heat transmission value is observed.

In an especially advantageous development of that method, the stream of hot gas is delivered from an outlet opening the diameter of which is approximately the same as the diameter of the beaker-like depressions and the distance of which from the upper side of the film is less than that diameter.

As a result of that measure, there is produced, surprisingly, a well-formed well the outer side of which rests over its entire surface against the inner wall of the beaker-like depression.

A further advantage is obtained in that method when the shaping block is temperature-controllable and has a temperature below the melting point temperature of the film, preferably about 100°C.

That measure has the effect that the wall thickness of the wells formed is approximately equal in the region of the heat-exchange surface.

It is also preferable in that exemplary embodiment for the plastics film to be a polycarbonate film.

The use of a polycarbonate film imparts sufficient mechanical strength to the plate, so that a very large number of wells can be introduced into the polycarbonate film. In addition, reaction vessels of polycarbonate, such as the wells formed in the plate, are inert with respect to the chemical and/or biochemical and/or microbiological reactions or substances in question.

Further advantages will be found in the description and the accompanying drawing.

It will be understood that the features mentioned above and the features that are still to be explained can not only be used in the combination indicated but can also be used in other combinations, or on their own, without departing from the scope of the present invention.

An exemplary embodiment of the invention is shown in the drawing and is described in greater detail in the description which follows.

Fig. 1 is a perspective view of a portion of the plate according to the invention with the wells open towards the top;

Fig. 2 shows the plate from Fig. 1 in a sectional view along line II – II in Fig. 1;

Fig. 3 is a diagram illustrating the method of producing the plate from Fig. 1;

Fig. 4 is a perspective view of a portion of a plate according to Fig. 1 being covered with a cover film;

Fig. 5 shows the covered plate from Fig. 4, with annular seams which are arranged around the wells and join the cover film to the plate, viewed in the direction of arrow V from Fig. 4;

Fig. 6 is a sectional part view of a welding die for creating the seams from Fig. 5;

Fig. 7 is a part view, seen from above along arrow VII in Fig. 6, of the welding die from Fig. 6;

Fig. 8 is a sectional side view along line VIII-VIII from Fig. 5 of the seam from Fig. 5.

Fig. 9 is a perspective view of a device for welding the covered plate from Fig. 4, in which a plurality of welding dies from Fig. 6 are used; and

Fig. 10 is a perspective view showing the use of the welded plate from Fig. 5 in conjunction with a thermal block, only of portion thereof being shown.

Fig. 1 shows a portion of a rectangular flexible plate 2 with one of its longitudinal edges 3 and one of its side edges 4. The plate 2, which is made, for example, of a rigid plastics film, has a flat upper side 5 and, parallel thereto, an underside 6. Its thickness, measured between the upper side 5 and the underside 6, is indicated

by reference numeral 7. As can be seen from Fig. 1, the thickness 7 of the plate 2 is small in comparison with its transverse measurements.

The plate 2 is provided with through-holes 9 and has wells 11 which are open towards the top. The wells 11 are arranged in rows 12 and columns 13, the rows 12 extending parallel to the longitudinal edge 3 and the columns 13 parallel to the side edge 4. The rows 12 and columns 13 are spaced apart from one another as shown by reference numerals 14 and 15, respectively. In the embodiment shown, the wells 11, which are circular in cross-section, have an internal diameter 16 which can be seen more clearly in Fig. 2. The row spacings 14 and the column spacings 15 are equal, the internal diameter 16 of the wells 11 of course being smaller than the row spacing 14 or the column spacing 15.

The wells 11 are arranged with their openings 18, which are surrounded by a rounded rim 17, in the plane of the upper side 5 of the plate 2. They have a wall 20 defining their interior space 19, which wall is formed in the manner of a beaker-like protuberance 21 and is located below the underside 6 of the plate 2 for each of the wells 11, which are identical to one another.

In the further course of the description, the term "towards the top/upwards" denotes the direction out of the interior 19 of the wells 11 through the opening 18 and the term "towards the bottom/downwards" accordingly denotes the opposite direction.

As can be seen more clearly in Fig. 2, the protuberance 21 has a hollow-cylindrical upper portion 22 and, formed integrally therewith, a hemispherical lower portion 23, the curved base wall 24 of which closes the well 11 towards the bottom. The upper side 5 merges directly into the inner surface of the interior space 19 of the well 11, in so doing forming the peripheral rounded rim 17, while the underside 6, forming a channel 27 around the protuberance 21, extends as the outer side 28 of the protuberance 21 substantially parallel to the curved inner surface 25. Webs 29, which separate the individual openings 18 from one another, run between the individual wells 11.

As can also be seen in Fig. 2, the base wall 24 has a thickness, indicated by reference numeral 31, which is measured between the inner surface 25 and the outer side 28. In the region of the hollow-cylindrical upper portion 22, reference numeral 32 denotes a correspondingly measured thickness at 32, which corresponds approximately to thickness 31. The wells 11 each have a volume 33 which is determined substantially by their depth, indicated by reference numeral 34, as well as by the internal diameter 16. The depth 34 is measured between the base wall 24 and a notional maximum filling level indicated by a dotted line at 35. The filling level 35 is at approximately the height at which the curved rim 17 merges into the perpendicular inner surface 25. On account of the surface tension and the associated curvature, particularly in the case of small volumes 33 the filling volume of the substances will be smaller than the maximum volume 33.

The wells 11 of the plate 2 described thus far serve for holding chemical and/or biochemical and/or microbiological substances which are stored or undergo a reaction in the wells. The volume 33 and the number of wells 11 per plate depend upon the substances contained in the wells 11. The row spacing 14 and the column spacing 15, as well as the internal diameter 16 and the depth 34, are largely stipulated by the volume 33. The thickness 7 of the plate 2 in the region of the webs 29 is so chosen that the plate 2, despite the closely arranged wells 11, has sufficient rigidity and does not sag when being transported with full wells 11. The thicknesses 31 and 32 of the wall 20 of the wells 11 are so chosen from the mechanical standpoint that the full wells 11 do not tear or even tear off.

In addition to the purely mechanical standpoint, the material from which the plate 2 is made and the thicknesses 31 and 32 of the wall are also selected from the physical standpoint. The thicknesses 31 and 32, which - as can be seen in Fig. 2 – are significantly smaller than the thickness 7, allow good transport of heat into and out of the interior space 19 of the wells 11. It is thus possible to cool or change the temperature of the substances in the wells very quickly, by bringing the entire outer side 28, heat-exchange surface 28', into contact with a temperature-control substance of the desired temperature.

In the exemplary embodiment chosen, the plate 2 is made of polycarbonate and has a thermal conductivity of $\lambda = 0.21$ W per Kelvin and metre. The thickness 7 is about 0.27 mm, and for the thickness 31 the following applies: $x = 0.04$ mm. The spacings 14 and 15 between the rows 12 and columns 13, respectively, are about 10 mm and the volume 33 of the wells 11 is: $V = 85$ µl. The size of the heat-exchange surface 28' corresponds to the outer side 28 and is: $A = 75\ mm^2$. In accordance with the formula:

$$W = \frac{A \cdot \lambda}{V \cdot x}$$

those numerical values result in a heat transmission value of about $4.5x10^3$ W/(K mm^3). It has been found that for such a heat transmission value the heat exchange through the wall 20 takes place so rapidly that the determining time factor is the heat conduction in the substances themselves.

The novel plate 2 thus makes is possible, for example, in a small space to carry out a large number of reactions in separate wells, it being possible to effect very good thermal control of the reactions through the wall 20 of the wells.

Furthermore, the material of the plate 2 can be so chosen that through the wall 20 of the wells 11 optical analysis process, such as, for example, absorption measurements or fluorescence measurement are possible. The material must for that purpose be transparent in the light wavelength range that is of interest, that is to say in that wavelength range it should exhibit neither appreciable absorption nor fluorescence emission.

A method of producing the plate 2 from Fig. 1 will now be described with reference to Fig. 3. The starting material is a thin foil 36, for example made of a polycarbonate, of thickness 7. The foil 36 is placed on a heat-controlled shaping block 37 in which there are provided blind bores 38, open towards the top, which are arranged like the wells 11 in rows 12 and columns 13. The blind bores 38 have a wall surface 40 surrounding their interior 38 which is smooth and enclosed. The

dimensions of the blind bores 38 are so chosen that they correspond to the external dimensions of the protuberances 21 to be formed; in the chosen example the blind bores have a diameter of about 6 mm and a depth of about 4 mm.

In the shaping block 37, which is made of metal, for example aluminium, there is provided a heating means (indicated diagrammatically at 43) by means of which the shaping block 37 is heated uniformly to 100°C. In the direction of the blind bores 38 there is arranged above the shaping block 37 an air nozzle 45 which is movable in the direction of arrow 46. The direction 46 extends parallel to the columns 13 or the rows 12, so that the air nozzle 45 can be positioned centrally above each individual blind bore 38. The direction 46 is also aligned parallel to the upper side 5 of the foil 36 applied to the shaping block 37, so that the distance between the air nozzle and the upper side 5 remains constant.

The air nozzle 45 emits a stream of hot air 47 at about 280°C which is discharged downwards from its outlet opening 48 at a rate of about 2-5 m/sec approximately perpendicular to the shaping block 37. The outlet opening 48 has a diameter of about 5 mm and is located 4 mm above the upper side 5 of the foil 36. The air nozzle 45 is successively positioned centrally over the individual blind bores 38 where they remain stationary for about 3 to 5 seconds. As a result of the stream of hot air 47 striking the upper side 5, the foil 36 is heated to an extent such that it is plastically deformable.

The stream of hot air 47 then blows the region of the foil 36 originally lying above the blind bore 38 into the interior 39 of the blind bore in question, that region gradually stretching and the original thickness 7 of the foil 36 being increasingly reduced in that region until finally the wall 20 of the well 11 that has been formed has the thickness 31 or 32 indicated in Fig. 2.

In Fig. 3, the right-hand well 11/1 is already finished, and the air nozzle 45 is located above the blind bore 38/2 in which the well 11/2 is just being formed. The base 24 of the well 11/2 has already moved partly into the interior 39/2 of the blind bore 38/2 and will then come to rest over the entire surface of the smooth inner

wall of the blind bore 38/2. As can be seen in Fig. 3, the web 29 between the wells 11/1 and 11/2 remains in the original thickness 7 of the foil 36. When the wells 11 are being formed, the enclosed air escapes without forming bubbles.

It is, of course, possible instead of using one air nozzle 45 to use a plurality of parallel air nozzles 45 the outlet openings 48 of which are arranged in the grid pattern of the columns 13 or rows 12. In that way, depending upon the number of air nozzles 45, all the wells 11 of a row 12 or of a column 13 can be produced simultaneously.

As already described above, the foil 36 consists of a polycarbonate of thickness 0.27 mm. Prior to the forming of the wells 11, the foil 36 is milkily opaque. It has been found, however, that with the hot air stream 47 at a temperature of 280°C and the shaping block 37 at a temperature of 100°C, the foil 36 becomes transparent in the region of the wall 20 of the shaped wells 11, as is required for the above-mentioned optical analysis methods. Adjusting the temperature of the shaping block 27 to 100°C, which is not necessary for the actual shaping of the wells 11, also results in the outer side 28 of the well 11 lying snugly against the wall surface 40 of the blind bore 38 in question. As a result, the outer side 28 of each well 11 likewise has a smooth and uniform surface, which is of great advantage for changing the temperature of substances contained in the wells 11. The protuberances 21 have almost identical contours, so that their heat-exchange surface 28' can be brought into direct contact with counter-surfaces formed correspondingly in the blind bores 38 without layers of air that would disrupt the thermal transfer. This is described again below with reference to Fig. 10.

Particularly when the volume of the wells 11 is small, the wells 11 should be sealed from the outer atmosphere. For that purpose, a cover plate 50 shown in Fig. 4 is provided which consists of a thin cover film 49. The cover film 49 is provided with through-holes 50 which are arranged in the same grid pattern as the through-holes 9 in the plate 2. The cover film 49 has a flat upper side 51 and, parallel thereto, an underside 52, which comes into contact with the upper side 5 of the plate 2 when the plate 2 is covered. The cover film 49 has a thickness 53,

measured between the upper side 51 and the underside 52, that is small relative to the traverse dimensions of the cover film 49. The cover film 49 is made, for example, of 0.1 mm thick polycarbonate.

When being applied to the plate 2, the cover film 49 is so oriented that the through-holes 50 are in alignment with the through-holes 9. In that way, the cover film 49 and the plate 2 can be simultaneously joined together and fixed to a support device in a manner that will not be described in detail.

It will be understood that instead of the through-holes 50 and the through-holes 9 it is possible to provide downwardly and upwardly projecting cylindrical pegs which engage in the through-holes 9 and the through-holes 50, respectively, when the cover film 49 is applied to the plate 2 and thus join the cover film 49 releasably to the plate 2.

The material preferably used for the cover film 49 is, as already mentioned, a polycarbonate 0.1 mm in thickness. Such a film is transparent in the wavelength range of interest for the optical analysis methods used and has only very low inherent fluorescence. The optical analysis methods can thus also be carried out from the top through the cover film 49; in particular, it is possible to measure the optical density of the substances contained in the wells 11 in a radiation process through the cover film 49 and the base wall 24 of the wells.

In the case of the preferred small volumes 33 of the wells 11, which are in the range of between 30 and 100 µl, the volume of solutions contained in the wells 11 can change as a result of condensation and/or evaporation effects. This is especially the case when it is necessary for the solutions to undergo frequent changes of temperature between high and low temperatures, as occurs in the polymerase chain reaction (PCR), a method often used for amplifying single nucleic acid strands.

In order to increase the sealing action of the cover film 49, the cover film 49 is joined to the plate 2 in the region of each well 11 by a closed annular seam 55

which surrounds the rim 17 of the well. It will be seen in Fig. 5 that each seam 55 defines a circular region 57 of the cover film 49 that covers the opening 18 of an associated well 11. In that way, each well 11 can be covered as it were with its own lid in the form of the circular region 57 which is joined by the seam 55 to the webs 29 surrounding the well 11 so that each well 11 is gas-tightly closed relative to the atmosphere and relative to the other wells 11.

For producing the individual seams 55, there is used, for example, a welding die 59 having a shaped end face 58, part of which is shown in Fig. 6. The welding die 59 has a solid-cylindrical base body 60 which carries at its upper end 61 an annular shoulder 62 formed integrally with the base body 60. The annular shoulder 62 defines a circular recess 63 which is concentric with the base body 60 and thus with the longitudinal axis 64 thereof, and carries the crown-shaped end face 58 which points away from the base body 60.

The end face 58, which surrounds the recess 63 like a crown, has shaping in the form of pyramids 65 arranged in rows, which pyramids are formed integrally with the annular shoulder 62 at their square base 66. The tips 67 of the pyramids 65 point away from the base body 60 in a direction parallel to the longitudinal axis 64 of the welding die 59.

Fig 7 shows a portion of the end face 58 in plan view in the direction of arrow VII in Fig. 6. As will be seen, the pyramids 65 are arranged in rows 68 and 69 which are displaced relative to one another by half the width of a pyramid base 66. The arrangement is such that between two rows 68/1 and 68/2, which extend parallel to one another and are not displaced relative to one another, there is a row 69/1 which is accordingly displaced relative to the rows 68/1 and 68/2 by half the width of a pyramid base 66. The row 68/2 is immediately adjoined, on the other side from the row 69/1, by a row 69/2 which extends parallel to the row 69/1 and is aligned laterally therewith.

Returning to Fig. 6, it will be seen that the welding die 59 is provided with a heater indicated diagrammatically at 71, by means of which the welding die 59, which is

preferably made of V2A steel, is heated to about 280°C. To make the seam 55, the heated welding die 59 is placed from above onto the upper side 51 of the cover film 49 which has been applied to the plate 2 so that its shaped, annular end face 58 surrounds centrally the rim 17 of the well 11 to be welded which is located under the cover film 49. The circular recess 63 has a diameter such that the tips 67 of the pyramids come to rest outside the rim 17 on portions of the cover film 49 located beyond the webs 29.

The square base 66 of the pyramids 65 measures 0.5 x 0.5 mm and the tip 67 of the four-sided pyramids 65 lies 0.25 mm perpendicularly above the pyramid base 66, that is to say two opposite sides of the pyramid encloses an apex angle of 90°. In the radial direction, up to three pyramids 65 are arranged one behind the other on the annular end face 58, so that the welding die 59 as a whole has an external diameter that is at least 6 base lengths of a pyramid 65 greater than the diameter of the recess 63.

The following method has proved satisfactory for welding a cover film 49, the thickness 53 of which is about 0.1 mm, to a plate 2, the thickness of which is about 0.27 mm: the cover film 49 is laid on the plate 2 from above so that it covers the wells 11 and the through-holes 50 are aligned with the through-holes 9. The welding die 59, which is heated to about 280°C, is placed from above, with its end face 58 foremost, onto the upper side 51 of the cover film 49 so that it is located centrally over a well 11 to be welded which is located underneath the cover film 49. The pyramids 65 on the end face 58 are then located with their tips 67, which may penetrate slightly into the material of the cover film 49, on the upper side 51 and heat the latter. The cover film 49 is in that way preheated for about 13 seconds by the honeycomb-like profile of the end face 58. The welding die 59 is then pressed downwards onto the cover film 49 by about 0.1-0.2 mm, so that each pyramid 67 penetrates into the cover film 49 which in turn penetrates into the webs 29 of the plate 2. The welding die 59 remains in that position for two seconds, then it is lifted away completely from the cover film 49.

The seam 55 so formed, which is a kind of welded seam, is shown in cross-section along line VIII – VIII in Fig. 5. The cooled seam 55 has the same shaping as that of the welding die 59. The pyramids 65 have pressed inverted-pyramid-shaped depressions 73 into the preheated upper side 51 of the cover film 49, which correspond in shape to the pyramids 65. Furthermore, the underside 52 of the cover film 49, in the region of its depressions 73, has been pressed into the upper side 5 of the webs 29, which has been preheated indirectly by the cover film 49, and has there formed depressions 74 which correspond to the depressions 73. In that way, a contact surface 75 has been formed between the underside 52 of the cover film 49 and the upper side 5 of the plate 2, which contact surface 75 is zig-zag-shaped in cross-section. As a result of that zig-zag shape, the contact surface 75 is larger than the support surface that was present before the welding between the underside 52 of the cover film 49 and the upper side 5 of the plate 2 in the intended region of the seam 55.

As a result of the action of heat of the pyramids 65, not only has the support surface been enlarged, but along the contact surface 75 the cover film 49 and the webs 29 have also been bonded to one another by welding. It has been found that such a seam 55 provides a good closure to the individual wells 11 which is not only impermeable to liquids but is also gas-tight, even in the event of frequent changes between high and low temperatures on the underside 6 or on the outer side 28. The seam 55 also withstands the customary mechanical stresses to which the closed plate 2 is exposed in everyday use in the laboratory, as well as the very small changes in shape and stresses associated with the changes in temperature.

During the welding operation described above, the circular portion 57 of the cover film 49 covering the opening 18 bulges upwards dome-like so that a closed plate 2 welded as described above has a lens-shaped dome 76 of cover film 49 over each well 11.

Because the dome 76 is formed only in the case of gas-tightly welded wells 11, however, it is at the same time a visual indication that the seam 55 produced has

provided a gas-tight closure for the well 11 in question. If the cover film 49 has no domes 76 after welding, either the welding operation was defective, for example in respect of the dwell times or the temperature of the welding die 59, or the penetration depth of the pyramids 65 into the upper side 51 was incorrect.

In the exemplary embodiment being described, the temperature of the welding die 59, the dimensions of the pyramids 65 and the penetration depth of the pyramids 65 into the upper side 51 of the cover film 49 are given merely by way of example for a cover film of polycarbonate 0.1 mm thick and a plate 2 of polycarbonate 0.27 mm thick. In the case of thicker polycarbonate foils, the penetration depth of the pyramids, which corresponds approximately to the thickness of the cover film, can be matched to the new thicknesses.

A further important factor for the success of the welding operation, in addition to the correct observation the dwell time of the welding die 49, firstly on the upper side 51 and then in the position in which it has been pressed into the upper side, is the depth by which the pyramids 65 penetrate into the material of the cover film 59. Although the above-described welding operation can be carried out manually, the yield of correctly located seams 55 is significantly increased by the use of a welding device 78 shown in Fig. 9.

The welding device 78 has a flat, rectangular base plate 79 and a flat head plate 80 which is arranged above the base plate 79 and has approximately the same transverse dimensions as the base plate 79. The head plate 80 is attached to the base plate 79 with the aid of four guide rods 81. Of the four guide rods 81, which are each screwed into the base plate 79 from above in the region of one of the four corners, the front right-hand guide rod 81/4 in Fig. 9 is shown broken away for reasons of clarity.

Between the base plate 79 and the head plate 80 there is provided a height-adjustable support plate 82, in the outer corners of which there are installed ball bushes 83 through which the guide rods 81 pass. As drive means for the height adjustment of the support plate 82, an electrically operated drive motor 84 is

attached by its flange 85 to the head plate 80 from above, on the other side from the support plate 82. The motor 84 has a motor shaft, indicated by reference numeral 86, which is connected to a ball screw drive indicated by reference numeral 87. The ball screw drive 87 is connected at the other end to the support plate 82 and serves for converting the rotary movement of the motor shaft 86 into the displacement movement of the support plate 82 along the guide rods 81.

On the other side from the ball screw drive 87, there is provided centrally below the support plate 82 a heating block 89 which is attached to the support plate 82 from below by means of four spacer bolts 90. The heating block 89, which is made of copper, fulfils the function of the heater (indicated by reference numeral 71 in Fig. 6) for the welding dies 59, of which three are shown in Fig. 9. The welding dies 59/1, 59/2 and 59/3 are installed from below in the heating block 89, on the other side from the spacer bolts 90, and their end faces 58 point downwards away from the heating block 89.

In the heating block 89 there is provided a blind bore 91 which extends almost completely through the heating block from right to left in Fig. 9, into which blind bore 91 there is inserted an electrically heatable heating cartridge which is not shown for reasons of clarity. The temperature of the heating block 89 is measured in a suitable way by a temperature sensor (not shown) and is supplied to a regulating circuit (likewise not shown) which in turn controls the heating cartridge. In a manner known *per se*, there is thus formed a closed regulation circuit by means of which the temperature of the heating block 89 is kept at a constant value, for example 280°C. By means of the spacer bolts 90, the heating block 89 heats the support plate 82, which can result in the ball bushes 83 becoming jammed on the guide rods 81. For that reason, the support plate 82 is provided with coolant bores 92 by means of which the support plate 82 is connected to a thermostat cooling circuit. In that way, the temperature of the support plate 82 is controllable by means of an external thermostat, irrespective of the temperature of the heating block 89, so that smooth-running displacement of the support plate 82 along the guide rods 81 is ensured.

Approximately in the centre below the heating block 89, the height of which above the support plate 82 is adjustable, there is provided on the base plate 79 an upwardly pointing holder block 93. The holder block 93 has, passing through it, a coolant bore 94 which, in the same way as the coolant bore 92 of the support plate 82, is connected to an external thermostat circuit which maintains the holder block 93 at a constant and adjustable temperature.

The holder block 93 has cups 95 which are open towards the top and which are designed to receive the protuberances 21 projecting downwards beyond the plate 2. The cups 95 therefore have the same dimensions as the blind bores 38 in the shaping block 37 (which can be seen in Fig 3) and are arranged in rows 12 and columns 13 in the same way as the wells 11.

A plate 2 has been placed onto the holder block 93 from above, the plate 2 having itself been covered by a cover film 49. A perforated mask 96, which engages over the holder block 93 from above on all sides, has been slipped over the cover film 49, the perforated mask 96 pressing the cover film 49 onto the plate 2, the wells 11 of which are turn pressed into the holder block 93. In the perforated mask 96 there are provided through-holes 97 which are aligned with the welding dies 59 and which are likewise arranged in rows 12 and columns 13 so that a hole 97 is aligned centrally over each well 11. For reasons of clarity, the perforated mask 96, the cover film 49 and the plate 2 are shown broken away and displaced relative to the holder block 93.

It will be understood that a hole 97 and a welding die 59 is provided for each well 11 of the plate 2.

For attaching the perforated mask 96 to the base plate 79, there are arranged on both sides of the holder block 93 two identical upwardly pointing bases 98, of which the right-hand base 98/2 is shown broken away. The base 98/1 has an upwardly pointing fixing bore 99 on which a fixing clip, which can be, for example, in the form of a spring clip or a latch, is fixed in order to press the perforated mask 96 downwards onto the holder block 93.

The fixing clip has been omitted in Fig. 9 for reasons of clarity.

The welding device 78 described thus far operates as follows: the support plate 82 is located in the raised starting position shown in Fig. 9. Once the perforated mask 96 has been removed from the holder block 93, a plate 2 to be welded is placed on the holder block 93 from above in such a way that the wells 11 come to rest with their protuberances 21 in the cups 95. The wells 11, the openings 18 of which point upwards, have already been filled with the desired substances and covered by a cover film 49, or they are now so filled and then covered with a cover film 49, which is so oriented that its through-holes 50 are in alignment with the through-holes 9 in the plate 2. The perforated mask 96 is slipped over the plate 2 so covered, its through-holes 97 coming to rest centrally over the wells 11. With the aid of the fixing clips provided on the bases 98, the perforated mask 96 is firmly pressed downwards onto the holder block 93.

The heating block 89 is heated to 280°C by means of the heating cartridge inserted in the blind bore 91. The welding dies 59, which are thermally conductively connected to the heating block 89, are also at that temperature. By means of the ball screw drive 87, the rotational movement of the motor shaft 86 of the drive motor 84 is converted into a downwardly directed movement of the support plate 82 guided by means of the ball bushes 83 and the guide rods 81. As the support plate 82 and therewith the heating block 89 is lowered, the welding dies 59/1 and 59/2 are moved from above into the associated holes 97/1 and 97/2, respectively, of the perforated mask 96. The transmission ratio of the ball screw drive 87 and the number of rotations of the motor shaft 86 are such that at the end of the downward movement of the support plate 82 the welding dies 59 come to rest with their end face 58, or the tips 67 of the pyramids 65, just on top of the upper side 51 of the cover film 49, as already described above.

In that position, in which the welding dies 59 preheat the cover film 49 and the plate 2 in the region of the seams 55 to be made, the welding device 78 stays for about 13 seconds. After that preheating time, the support plate 82 is gradually

moved further downwards by the motor 84 by means of the ball screw drive 87 towards the holder block 93, so that the pyramids 65 on the end face 58 of the welding dies 59 penetrate the cover film 49 and press it into the webs 29 of the support plate 2. After a further 2 seconds, the motor 84 is so controlled that its motor shaft 86 turns in a direction opposite to the previous direction of rotation and thus the support plate 82 and therewith the heating block 89 and the welding dies 59 are raised into the starting position shown in Fig. 9 again by means of the ball screw drive 87.

After the fixing clips have been released, the perforated mask 96 can be removed and the welded plate 2 as shown in Fig. 5 is removed from the holder block 93.

The next plate 2 is then placed on the holder block 93 and the welding process starts again from the beginning.

For many experiments it is necessary to keep the substances contained in the wells 11 at low temperatures and to prevent then from being heated during the welding process just described. For that purpose, the holder block 93 and therewith its cups 95 are thermostatically maintained by means of the coolant bore 94 at the temperature required by the substances in question, for example at 10°C. The wells 11 lie with their heat-exchange surface 28' closely against the inner wall of the respective cups 95, so that by virtue of the small thickness 31 of the wall 20 of the wells 11 the substances located in the wells 11 are kept at the same temperature as the holding block 93 itself. Any heat supplied to the substances during welding is instantaneously dissipated through the wall 20 into the holding block 93 by virtue of the good heat transfer.

In that way, with the aid of the new welding device 78 it is also possible for substances that respond very sensitively to temperature fluctuations to be enclosed by welding in the wells 11 of the novel plate 2 . As a result, it is possible for large numbers of temperature-sensitive substances or solutions or highly infectious substances to be packed gas-tightly into an extremely small space to an extent not known hitherto. The substances can be, for example, pre-prepared

reaction solutions for biochemical and/or microbiological test procedures that are delivered to the user already in portioned and welded form in the novel plates 2. The substances to be tested by the user can be introduced, for example, into the test solutions located in the wells 11 by piercing the domes 76 covering the openings 18 of the wells 11 from above with a thin cannula. The substances to be tested are then injected into the test solutions located in the wells 11.

After withdrawal of the cannula, which is, for example, a syringe in everyday use in a laboratory, a capillary-like channel remains in the dome 76. Exchange of moisture with the surrounding atmosphere cannot take place by means of that channel, so that the volume of the substances or solutions contained in the gas-tightly welded wells 11 is not changed by condensation or evaporation effects.

Usually, however, the wells 11 of the novel plate 2 are filled on site, for example in the chemistry laboratory, and closed gas-tightly with a cover film 49 using the novel welding device. The fixed grid pattern of columns 13 and rows 12 makes it possible to fill a plurality of wells 11 simultaneously using a multiple pipette known *per se*.

Fig. 10 shows a plate 2 with gas-tightly closed wells 11 which contain, for example, solutions the course of reaction of which can be influenced by means of their temperature. The solutions have either been introduced into the wells 11 on site or were already contained in the plate 2, supplied welded, or have been inoculated subsequently by the user with the substances, for example DNA molecules, to be tested.

The plate 2 so prepared is inserted from above into a thermal block 101 having blind bores 102 which are open towards the top and which serve for accommodating the beaker-like protuberances 21. The blind bores 102 have the same shape as the blind bores 38 in the shaping block 37 used for the production of the plate 2. After insertion of the protuberances 21 into the blind bores 102, the inner wall 103 thereof lies directly against the heat-transfer surface 28' of the protuberances 21. There are therefore no layers of air between the outer side 28

and the inner wall 103 acting as the counter-surface 103' that would disrupt the transfer of heat between the thermal block 101 and the interior 19 of the wells 11.

In the thermal block there are also provided threaded bores 104, which are open towards the top and which are in alignment with the through-holes 50 and 9 in the cover film 49 and in the plate 2, respectively, when the plate 2 is inserted in the thermal block 101. Screws 105 are screwed from above into the threaded bores 104 through the through-holes 50 and 9 and thus the plate 2 closed by the cover film 49 is securely joined to the thermal block 101. The thermal block 101 comes to rest with its upper side 106 tightly against the underside 6 of the plate 2, and the protuberances 21 are pressed by their heat-exchange surfaces 28' securely onto the inner wall 103 of the blind bores 102.

By virtue of the smooth surface of the outer side 28, which is in direct thermal contact with the inner wall 103, and by virtue of the described high heat transmission value, the solutions located in the wells 11 assume the temperature of the thermal block 101 within only a few seconds. If the solutions are to be stored, for example, for a prolonged period at a low temperature, the temperature of the thermal block 101, which is made of a metal having good thermal conductivity, is maintained, for example at +4°C, by means of a thermostat connected thereto.

When the reaction in the solutions is to be started, the thermal block 101 is heated in a suitable manner to the reaction temperature of the solutions, which on account of the good thermal transfer follow the change in temperature of the thermal block 101 almost immediately. The change in the temperature of the thermal block 101 itself can be effected in a manner known *per se* by immersing the thermal block 101 in water baths of different temperatures, by bringing it into heat-conductive contact with pre-heated further metal blocks or by moving it along a metal rail on which a temperature gradient has been established.

The metal rail with the temperature gradient, in particular, facilitates the cyclical change in the temperature of the thermal block 101 and thus the temperature of

the solutions in the wells 11. For carrying out the polymerase chain reaction in the wells 11, the thermal block 101 is, for example, first maintained at 37°C for 60 seconds, then at 72°C for 120 seconds, then at 94°C for 60 seconds and then at 37°C again for 60 seconds, and so on.

A decisive factor for the progress of the polymerase chain reaction is the time required to bring the solutions to the individual temperatures. While a typical reaction sequence in the known plastics reaction vessels takes more than 10 hours and is usually carried out overnight, the reaction using the novel plate 2 is complete in less than 4 hours. Such an experiment can therefore now be prepared, performed and analysed in the course of one day.

When the test sequence is complete, the solutions are at least to some extent used further, for example analysed using a separating gel. For that purpose, the dome 76 is pierced with the syringe indicated by reference numeral 107 in Fig. 10 and a portion of the solution is removed. After withdrawal of the syringe 107, the solution remaining in the well 11 can be stored, for example in the way described above. Although the hole produced in the dome 76 during removal does not result in any appreciable moisture exchange, it can be sealed again afterwards with adhesive film.

Finally it should be mentioned, solely for the sake of completeness, that the transverse dimensions of the novel plate 2 as well as the row and column spacings 14 and 15, respectively, depend substantially on the required filling volume 33 of the wells 11. The thermal blocks 101, holder blocks 93 and shaping blocks 37 are each matched to those spacings. In all cases, however, the thickness 7 of the film 36 is so chosen that the wells 11 in the finished plate 2 have a base wall 24 having a thickness in the region of 0.04 mm, so that the heat transmission value has the required high value.